

## Truth in Numbers

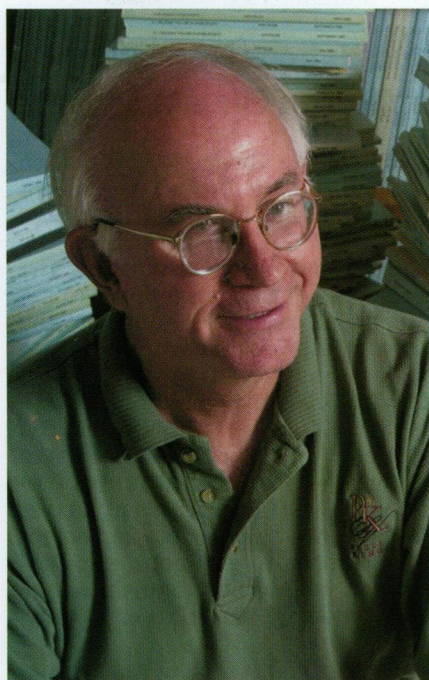
Along one wall of Joseph K. Haseman's office stands a floor-to-ceiling bookcase lined with identical powder blue books. These are Haseman's pride and joy: 500 technical reports representing rodent carcinogenicity assays conducted over the past 20 years for the National Toxicology Program (NTP). As director of statistical consulting for the NIEHS, Haseman is responsible for the statistical integrity of studies done for the NTP as well as other programs within the institute. His 30 years' experience in experimental design, data analysis, and interpretation of experimental findings has made him a valued expert. "Joe Haseman has done more than any other person to ensure that the NTP rodent studies are evaluated in a comprehensive, consistent, and objective manner," says John Bucher, deputy director of the NIEHS Environmental Toxicology Program.

Haseman's specialty is extracting the truth from the voluminous data produced by NTP bioassays. As the saying goes, there are lies and there are damned lies, and then there are statistics. Unless one understands what they are seeing in the numbers and is committed to objectivity, it is just as possible to draw misleading conclusions from tumor counts as it is from other areas of statistical analysis. Haseman analyzes data for 80 different NIEHS scientists covering all aspects of environmental health, but his principal focus has been on cancer research, specifically on rodent bioassays.

Over the years, Haseman and colleagues have been involved in a number of high-profile studies of potential carcinogens. These include studies on vitamin C, sodium fluoride, talc, and electromagnetic fields. These studies have identified a number of chemicals, including benzene and 1,3-butadiene (used in the manufacture of synthetic rubber and certain resins), as potent rodent carcinogens. Epidemiology studies have since confirmed the carcinogenicity of these two chemicals in humans as well.

### Getting at the Truth

NTP studies are designed to characterize and evaluate the toxicologic and carcinogenic potential of selected chemicals in laboratory animals. In a typical study, 50 male and female rats and mice are exposed to three different dosages of the chemical of concern. A control group is also monitored. After two years, a pathologist necropsies the animals and diagnoses tumors at various sites. A statistician



**Number wizard.** Joseph K. Haseman analyzes data for 80 different NIEHS scientists and has helped produce over 500 technical reports, which are used by regulatory agencies to perform risk assessments.

then evaluates the data to determine the likelihood that any increased tumor incidence is chemically related. Following this evaluation, the research team then characterizes the strength of the evidence of the chemical's carcinogenicity ("clear evidence," "some evidence," "equivocal evidence," or "no evidence").

The procedure seems straightforward. But assessing the true effect of a chemical is not simply a matter of counting tumors; a statistician's knowledge of background tumor rates and confounding variables provides a key perspective in this interpretive process. "Because of the many tumor types and sites examined and the use of multiple dosage groups and sex-species combinations, statistically significant tumor increases can and do occur by chance," Haseman says. "The question then becomes how to take into account the large number of statistical comparisons being made."

NTP study teams do not directly perform risk assessments for the chemicals they analyze. Rather, their findings are written into reports—the infamous blue books—which are then used by regulatory bodies such as the U.S. Environmental Protection Agency to perform these assessments. Risk assessments may then lead to regulations setting permissible levels of exposure or to the chemical's being banned altogether.

Each time NTP researchers analyze a

data set, the information is put into a massive database. By analyzing this database over the years, Haseman and his colleagues have been able to estimate the frequency with which false positive outcomes may occur, and they take this into account when interpreting experimental findings. "Examples of why statistically significant results may be discounted include lack of a dose-response relationship, an abnormally low concurrent control rate, or dosed-group tumor rates that are within the normal control range," Haseman says. "Statistics is an important tool, but you also have to use scientific judgment in interpreting the statistical findings."

By periodically reviewing the NTP database, Haseman and colleagues have also been able to observe and monitor changes in tumor rates over time. Understanding what causes these changes can lead researchers to take measures to reduce the incidence of background tumors and improve the health of the animals as well as the accuracy of the tests. For example, the researchers observed that certain tumors, such as liver tumors in mice, occur with greater frequency in heavier animals. They also noticed that over time, average body weights of test animals were increasing, contributing to a higher incidence of background tumors and a reduction in survival. In response to these findings, NTP researchers changed the rodent diet to reduce body weight and thus increase survival and reduce the incidence of background tumors.

Sometimes, exposure to suspected carcinogens results in a decrease in tumor rates. Here again, Haseman's extensive knowledge of the NTP database led to a discovery of one mechanism of action. Observing a reduction among Fischer 344 rats of mononuclear cell leukemia after exposure to a certain class of chemicals, Haseman dug back into the NTP database and noticed a clear association between a reduction in this illness and damage to the animal's spleen. Haseman consulted with NTP pathologist Mike Ewell, who informed him that removal of the spleen was known to reduce leukemia rates in Fischer 344 rats. Subsequent investigations showed that the chemicals of concern were damaging the rats' spleens in a way that mimicked removal, thereby reducing the incidence of leukemia.

### Blazing New Trails

Haseman's periodic reviews of the NTP database, written up and published in the



journal *Toxicologic Pathology*, are highly valued by other researchers in the field. In 1997, as part of its twentieth-anniversary celebration, the journal recognized a 1984 article by Haseman and coauthors James Huff and Gary Boorman, "Use of Historical Control Data in Carcinogenicity Studies in Rodents," as one of the 10 most frequently cited articles in the journal's history.

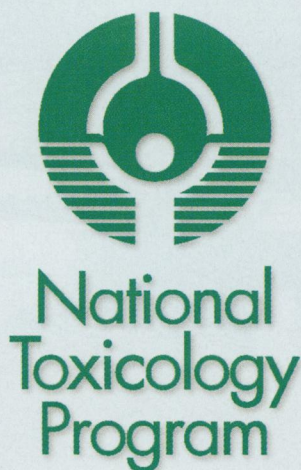
Recently, Haseman and colleagues have been refining the use of transgenic animals developed at the NIEHS for carcinogenicity studies. One of the benefits of these animals is that they evidence chemical-related carcinogenic effects more quickly than the strains used in the standard rodent bioassay. Using transgenic animals dramatically shortens the time frame required for completing the studies and reduces the number of animals that must be used. Transgenic mouse studies typically last only 6 months and usually use only 15–20 animals per group, compared with 2 years and 50–60 animals per group for the standard rodent bioassay.

However, the unique characteristics of the transgenic animal have also required new statistical methodologies. For instance, for some transgenic lines, the main tumor of interest is the skin papilloma, which can be observed directly rather than requiring a necropsy. A multiplicity of these skin papillomas can occur within the same animal over time.

In a standard rodent bioassay, the end point of analysis would be whether or not the animal developed a tumor, but for a transgenic mouse model, the end point of analysis is how many tumors the animal has and over what time period they develop. To accurately evaluate this response, Haseman and colleague David Dunson have developed new mathematical models that take into account both tumor multiplicity and the time course of tumor occurrence.

Haseman is frequently called upon to represent the institute in evaluations of new testing methods such as the Frog Embryo Teratogenesis Assay–*Xenopus*, or FETAX. Haseman takes pride in offering an unbiased analysis. "The developers of these tests want them adopted, sometimes very badly," he says. "I see it as my job to make certain that the statistical methodology utilized in these new models is appropriate and that the data are correctly evaluated and interpreted."

Although eligible to retire after more than 30 years of government service, Haseman is in no hurry. "I really enjoy my job, and the people at the institute are great to work with," he says. "I am one of those unusual individuals who loves working with numbers and large databases, and I certainly have plenty of opportunity for this at the NIEHS. As long as my job continues to be fun, I may be around for a long time." —John Manuel



## NTP May Test Cell Phones

At its 30 June 2000 meeting, the Executive Committee of the National Toxicology Program (NTP) approved a recommendation for the NTP to study radio frequency radiation emissions from wireless communication devices, which include cellular phones. The NTP is currently evaluating the need, feasibility, and scope of a possible testing program.

The recommendation came from the Food and Drug Administration, which regulates electronic products that emit radiation. The administration noted in its nomination that in the United States alone over 80 million people currently use portable communication devices, with about 25,000 new users daily.

Cell phones use low-level microwave radiation to communicate between the caller and a base station. If tissues are exposed to strong enough microwave fields, the rise in tissue temperature can cause well-documented thermal effects such as cataracts, deep-tissue burns, and heat stroke. Cell phones are currently required to meet exposure guidelines established by the Federal Communications Commission to protect against acute injury from thermal effects. However, nonthermal effects, which occur at much lower levels of exposures, are neither well established nor, consequently, protected against through regulation. Some research has suggested that such microwave radiation may influence DNA synthesis and tumor growth through unspecified ways.

It is widely agreed by scientists that more research is needed before any firm conclusions can be drawn regarding the human health effects of cell phone use. Scott Masten, head of the Office of Chemical Nomination and Selection, which coordinates and reviews nominations for NTP studies, says, "The public health concern and public health awareness about potential hazards of cell phone use is very clear. There's also a great deal of research effort globally on the health effects of radio frequency radiation emissions from wireless communication devices. Just the number of people exposed worldwide would make it a potential public health problem if any adverse effects were to be identified." —Susan M. Booker



**The appeal of transgenics.** Transgenic mouse studies typically last only one-fourth as long and require only one-third the number of animals, compared to standard rodent bioassays.